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THE VALUE OF VIRULENT SALT SOLUTION IN THE PRODUCTION OF ANTIHOG-CHOLERA SERUM BY THE INTRAVENOUS METHOD *

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Craig¹ first suggested the use of virulent salt solution in the manufacture of antihog-cholera serum. Its use also received attention from Robbins.² Their work indicates clearly that salt solution made virulent by passing through the peritoneal cavity of virus pigs can be injected subcutaneously into immune hogs in sufficient quantities to produce a potent antiserum. The intravenous method of serum-production is more widely used, but up to this time no systematic attempt has been made to use virulent salt solution in the course of hyperimmunizing by this method.

The necessity of employing large amounts of virus in the production of antihog-cholera serum of high potency by the subcutaneous method has prevented its general adoption. The cost of producing antihog-cholera serum by the intravenous method of injection, aside from other desirable features, is much less. This is quite generally conceded inasmuch as it requires about one-half the volume of blood virus if an immune animal is to be rendered hyperimmune by an initial dose of virus. Since the cost of serum is dependent upon the cost of the virus, any method which will increase the quantity of virulent material to be obtained from a single virus pig is therefore a factor in reducing the cost of serum. No doubt large quantities of virus are retained in the bodies of virus-producing animals, which under the present method are cremated. Attempts to remove the virus from the musculature and organs in sufficient quantities to render an animal hyperimmune following injection have not been consistently successful thus far.

There exist no means of determining the degree of virulence of a given virus other than that indicated by illness following its injection into susceptible shoats and by the potency of the antiserum produced by its use in hyperimmunization. If by the use of virulent salt solution

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¹ Bull. Purdue Experiment Station, 140.

² Jour. Infect. Dis., May, 1913, 12, p. 335.

in the intravenous method an antiserum of proven protective power can be produced, but little objection can be raised to the use of this method.

The work we wish to describe was conducted according to the ideas of Craig and of Robbins, departing from their technic in that we used the salt virus intravenously. They found, after extensive experiments, that virulent salt solution of hyperimmunizing value was best obtained, for the subcutaneous method, by the intraperitoneal injection of 25 to 30 c.c. of 0.85% salt solution per pound weight of the virus pig. This was allowed to remain in the peritoneal cavity for 5 to 6 hours. In our work 25 c.c. per pound weight of sterile 0.9% salt solution were injected into the peritoneal cavity of virus pigs at least 5 hours before killing, when the salt solution was removed aseptically. By the injection of salt solution the volume of blood obtained is considerably increased, approximately 10 to 20%. The salt solution is recovered in 40 to 70% of the volume injected. Table 1 indicates the approximate increase in the amount of virus obtained, with the relative amounts of salt solution administered and regained in a series of 11 pigs, the figures referring to quantities in cubic centimeters.

TABLE 1

INCREASE IN AMOUNT OF BLOOD VIRUS OBTAINED BY INTRAVENOUS INJECTION OF SALT SOLUTION, AND THE RELATIVE AMOUNTS OF SALT SOLUTION INJECTED AND REDRAWN

Pig (Weight 100-110)	Virus Blood Drawn	Salt Solution	Salt Solution Redrawn	Estimated Approximate Increase in Blood Virus	Total
1.....	1,800	2,500	1,200	200	3,000
2.....	1,600	2,500	1,600	400	3,200
3.....	2,150	2,500	1,600	550	3,750
4.....	1,800	2,500	1,100	600	2,900
5.....	1,950	2,500	1,600	350	3,550
6.....	2,150	2,500	2,000	550	4,150
7.....	2,000	2,500	1,950	400	3,950
8.....	2,400	2,500	1,650	800	4,050
9.....	1,800	2,500	1,700	200	3,500
10.....	1,900	2,500	1,500	300	3,400
11.....	2,000	2,500	None	400	2,000
Total.....	21,550	27,500	15,900	4,750	37,450

The salt virus and blood virus were first used for hyperimmunizing in 3 different proportions, namely:

Blood Virus		Salt Virus	Amount Injected
1 part	+	3 parts	7 c.c. per lb.
2 parts	+	2 parts	7 c.c. per lb.
3 parts	+	1 part	7 c.c. per lb.

The antisera yielded by all three of the animals indicated, proved to be protective when tested on susceptible shoats. Table 2 gives the results of the test on the sera produced in this way.

TABLE 2
RESULTS OF TESTS WITH ANTISERA MADE WITH VARYING AMOUNTS OF SALT VIRUS
AND BLOOD VIRUS

Weight of Pig	Dose Virus, c.c.	Dose Serum, c.c.	Temperatures																	Remarks
			May																	
			15	16	17	18	19	20	21	22	23	24	25	26	27	28	29			
50	2	20	102	102	102	102	103	103	102	102	103	102	102	102	...	...	...	Serum produced with ½ salt virus intravenously		
60	2	20	102	102	103	103	103	102	102	101	102	103	103	103	...	...	...			
75	2	20	102	102	102	102	102	102	102	102	102	102	102	102	...	...	...			
60	2	20	102	102	103	103	102	101	102	102	103	102	102	102	...	...	...	Serum produced with ½ salt virus subcutaneously		
60	2	20	102	102	102	102	102	101	102	102	102	102	102	102	...	...	...			
60	2	20	102	102	102	102	102	101	102	102	102	102	102	102	...	...	...			
60	2	20	103	103	103	103	103	102	102	102	102	102	102	102	...	...	...	Serum produced with ¾ salt virus intravenously		
65	2	..	102	102	102	102	102	102	103	103	103	103	104	106	106	105	105			
60	2	..	101	102	102	102	102	102	103	104	104	104	104	104	104	104	104			
			3	1	4	3	2	1	1	2	3	4	5	7	5	6	4	Controls. Chronic hog-cholera		

In subsequent experiments the following method was used: Immunes weighing from 150 to 300 pounds were injected with a mixture of equal parts of virus and virulent salt solution at the rate of 7 c.c. per pound body weight. Some tests were carried out in which the salt virus and blood virus were mixed without particular attention to the volume of either. The mixtures used varied from straight blood virus, in those pigs in which by error the salt solution was not aseptically drawn or was otherwise discarded, to equal proportions of each, but in most cases the salt mixture used was 60% blood virus and 40% salt virus. The serum resulting from hyperimmunizing in these proportions protected susceptible shoats against 2 c.c. of virus. The use of salt virus and blood virus as drawn from the virus pig in the routine production of antihog-cholera serum by the intravenous method is more practical than the use of other proportions, as it eliminates handling the salt and blood virus separately.

The antisera obtained from hyperimmunes of one series were mixed. To determine the potency of this mixture, we tested it on susceptible shoats weighing 50 to 100 pounds, as described in Table 3. (Dr. K. L. Pontius had charge of the testing of all experimental sera.)

TEST 1

Weight of Pig	Dose Virus c.c.	Dose Serum c.c.	Temperatures										Remarks	
			April						May					
			25	26	27	28	29	30	1	2	3	4		5
100	2	30	102	102	102	102	102	102	103	104	104	103	102	} Serum manufactured by hyper-immunizing immune hogs with ½ salt and ½ blood virus
100	2	30	102	102	102	102	103	102	102	102	103	103	102	
100	2	40	102	102	102	102	102	102	103	103	103	102	102	
100	2	28	103	103	102	102	102	102	103	103	104	103	103	
100	2	50	102	102	102	102	102	102	102	102	103	103	102	
100	2	50	101	102	102	102	102	102	103	103	102	102	102	
100	2	..	102	102	102	102	103	104	104	104	104	104	105	
100	2	..	102	103	103	104	104	104	104	104	104	105	105	
			2	3	4	2	3	4	4	5				

TEST 2

Weight of Pig	Dose Virus c.c.	Dose Serum c.c.	Temperatures													Remarks
			April							May						
			23	24	25	26	27	28	29	30	1	2	3	4	5	
100	2	25	102	102	102	103	103	102	102	102	103	102	103	102	102	Serum manufactured by hyperimmunizing immune hogs with salt virus and blood virus mixed just as drawn from cholera pigs
90	2	25		1				3	2	2		2		3	2	
100	2	20	102	101	101	102	102	102	102	102	102	102	102	102	102	
			1	2	3	4	4	3		3	4	4	4	2		
98	2	20	101	102	102	102	103	102	102	102	102	103	103	103	102	
			1			3			1	6	5	4			1	
80	2	15	102	102	102	103	103	102	102	102	103	103	103	102	102	Controls
				2	4	2	2		2	4	4	1			4	
76	2	15	102	102	102	104	104	103	103	103	103	103	102	102	102	
						2				1		2	1	3	3	
75	2	..	101	102	102	103	103	102	102	102	103	103	103	102	102	
			2	1	1	4	2	4	3	4	1	4	2	4	3	
75	2	..	102	102	102	104	104	104	104	104	Died.					
			1	3	4			2	1	2	5					
75	2	..	102	102	102	104	104	104	105	106	105	Died.				
			4	3	3	2	1		4		1					

TEST 3

Weight of Pig	Dose Virus c.c.	Dose Serum c.c.	Temperatures															Remarks
			May															
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
58	2	25	102	102	102	102	102	102	102	102	102	103	103	102	103	104	104	Serum manufactured by hyperimmunizing Immune hogs with salt virus and blood virus mixed as drawn from cholera pigs
60	2	25	102	102	103	102	103	102	103	102	103	103	103	104	104	104	102	
60	2	20	103	102	102	103	103	103	103	102	102	103	102	104	103	103	103	
60	2	20	102	102	102	102	102	102	102	102	103	103	103	103	103	104	104	
62	2	15	103	103	103	103	103	103	103	102	102	102	103	103	103	103	103	
65	2	15	102	102	103	102	102	102	102	102	102	104	103	103	104	103	104	
62	2	..	102	102	103	103	103	103	104	104	104	Died.....					Controls	
62	2	..	102	103	103	103	103	103	104	104	103	Died.....						

TABLE 3.—Continued
TEST 4

Weight of Pig	Dose Virus c.c.	Dose Serum c.c.	Temperatures																				
			May																				
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
60	2	15	102	102	102	102	102	102	102	102	102	102	102	102	104	104	103	103	102	102	101	101	
65	2	15	102	102	103	103	103	103	103	103	102	102	102	102	102	104	103	102	102	103	100	101	
54	2	20	103	102	103	102	102	102	102	102	103	103	102	102	104	103	103	104	104	104	104	102	102
55	2	20	102	102	102	103	103	103	102	102	102	102	103	102	103	103	104	104	104	104	103	102	102
60	2	25	102	102	103	103	102	102	102	102	102	102	102	102	102	103	104	103	103	104	103	102	102
65	2	25	102	102	102	102	103	102	102	102	102	102	102	102	102	103	103	103	103	103	102	103	102
54	2	..	102	101	102	103	104	104	105	Died	Controls												
68	2	..	103	102	102	103	103	104	106	Died													

Serum manufactured by hyperimmunizing immune hogs with salt virus and blood virus as drawn from cholera pigs.

The objection which may possibly be raised to the use of virulent salt solution is that the blood virus is weakened by dilution. This is possible, but in view of the fact that the serum produced by hyperimmunizing with mixtures of salt virus and blood virus protects susceptible shoats, such objections are not fundamental. In laboratories which make a practice of testing all serum before it is distributed for use in the field, the salt solution method has proved a perfectly safe and an economical procedure; that is, in the Indiana and Michigan state laboratories. We do not contend that the salt virus is as virulent as blood virus, but that a salt solution does contain hog-cholera virus after remaining for a time in the peritoneal cavity of a virus hog. Virulence is no doubt acquired by the salt solution as a result of the phenomenon of osmosis, the interchange of substances occurring between the lymph spaces and blood vessels of all the abdominal viscera and the salt solution. It is possible that if a higher concentration of common salt were introduced into the peritoneal cavity, more virulence would be acquired by the salt solution.

The more economical production of antiserum warrants the use of salt virus and blood virus mixed in the intravenous method as well as the subcutaneous method. Assuming that the amount of blood secured from a virus pig is 10 to 15 c.c. per pound body weight, we find that the average pig used for production of virus yields approximately 200 to 500 c.c. more blood virus by volume when salt solution is

injected into the peritoneal cavity. Including blood virus and salt virus, the volume of total virus solution is increased from 75 to 80%. The mixture of salt virus and blood virus must be used in larger doses in hyperimmunizing, but the difference between 5 c.c. per pound weight of blood virus and 7 c.c. per pound weight of blood and salt virus mixed does not offset the advantage gained. In these different series, containing 3 or more immunes hyperimmunized by this method, the serum resulting proved to be protective when tested on susceptible pigs, and this fact is suggestive of the value of this method of producing antihog-cholera serum.